A CYTOLOGICAL STUDY OF THE ORIGIN OF MELANOPHORES IN THE TELEOSTS [1]

DAVID C. SHEPHARD

Department of Zoology, University of Chicago, Chicago 37, Illinois

Conclusive evidence for the neural crest origin of all teleost melanophores does not exist despite the growing body of evidence that the neural crest does give rise to melanophores in many fish species. Extirpation, explantation, and transplantation experiments by a number of investigators on a variety of species definitely indicate that the neural region of teleost embryos is a source of future melanophores (Lopashov, 1944; Humm and Young, 1956; Kajishima, 1958). Descriptive findings, from one of the first suggestions that melanophores stem from neural ectoderm (Borcéa, 1909), to the recent extensive work of Orton (1953), lend further support to the concept that the neural crest is a source of melanophores in fish. In fact, a tabulation of the data from the literature cited in the present paper shows some sixty species of fish, representing more than twenty families and ten orders, in which there is some evidence for the neural crest origin of melanophores; however, none of this work constitutes evidence that the crest is the sole source of these cells. Conversely, Trinkaus (1951) has removed the entire embryo at very early stages, and the remainder of the egg produced a normal complement of melanophores simultaneously with their appearance in unoperated eggs. The species used in his study was *Fundulus heteroclitus,* of which Stockard (1915) has given a detailed description of the origin of melanophores from the extra-embryonic germ ring. Stockard's observations have been supported by other authors (Gilson, 1926; Russel, 1939). Further evidence that the germ ring may give rise to melanophores is contributed by the experimental studies of Oppenheimer (see 1949), although she does not agree that this structure is a normal source of melanophores. A search of the cited literature yields evidence for this secondary site of pigment cell origin in only *F. heteroclitus* and possibly *Epiplatys fasciolatus* (Oppenheimer, 1938). Even *Fundulus majalis* seems to lack these cells, judging from the description in Bancroft (1912). These exceptional pigment cells are found largely if not solely in the extra-embryonic yolk sac membranes of a very few species of cyprinodont fishes, and in *F. heteroclitus* they all eventually encircle the blood vessels of the yolk sac—a phenomenon, which is unusual and possibly unique among fish embryos (Stockard, 1915).

There are still numerous problems concerning the origin of fish melanophores, however. Fish do not have a morphological neural crest as seen in the higher vertebrates. The experiments cited above involved removal of pieces of the ectoderm plus some of the underlying neural keel, and hence are not truly analogous with removal of the neural folds in higher forms. The descriptive data are con-

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fined to reports of the melanophores or their immediate precursors first being seen in the "neural region." Newth (1951, 1956) has studied the crest and its derivatives in the lamprey. He describes the crest as a histologically distinct region between the keel and the overlying ectoderm. Kajishima (1958) mentions very briefly that he sees a similar region in the goldfish, but he does not figure it. There appears to be no published study identifying the neural crest of teleosts and determining its extent and time of migration. Goodrich (1950) is even led to state (p. 18) that "there does not at present appear to be available critical evidence to demonstrate the origin of fish chromatophores from the neural crest. . . ."

Concerning the extra-embryonic origin referred to above there is even less unanimity. Many of the descriptions predate DuShane's (1935) demonstration of the neural crest origin of amphibian pigment cells. Oppenheimer and Trinkaus have worked on this problem more recently, and Trinkaus (1951) contends there is a normal extra-embryonic source of pigment cells in *Fundulus heteroclitus*, while Oppenheimer (1949), working on the same species, attributes her results to a "bedeutungsfremde Selbstdifferenzierung" of cells in an abnormal situation.

The object of the present investigation is to describe the neural crest of several species of teleosts and to provide some information on its time of migration. The yolk sac melanophores of *F. heteroclitus* are identified at an early stage and followed throughout their differentiation to demonstrate more conclusively that in this species, and possibly in a few others, there is normally an extra-embryonic source of melanophores.

Materials and Methods

The embryos of several species of fish were used for this study. Much of the work on the neural crest was done on the zebra fish (*Brachydanio rerio*), a tropical cyprinid which is easily raised and bred in the laboratory. Its eggs are small (0.6 mm. in diameter) and transparent enough for observation with the phase microscope. The egg is reasonably good for histological work, too, since the chorion is easier to remove than those of most fishes, and it offers less difficulty in sectioning than the larger eggs.

The eggs of *Fundulus heteroclitus*, a cyprinodont, and those of the cunner (*Tautogolabrus adspersus*), a labrid, were studied at the Marine Biological Laboratory in Woods Hole, Massachusetts. *Fundulus* has an egg which is approximately 2 mm. in diameter, large enough for some experimental work, but sufficiently transparent to allow good observation of the cells on the yolk sac in the living egg. The cunner has a smaller egg, less than 0.5 mm. in diameter, and it has been described as "glass clear." It is pelagic and too delicate for most kinds of work.

Several other tropical fish were used briefly. Their taxonomy and handling were based mostly on Axelrod and Schultz (1955). The methods used with *Fundulus* and the cunners were taken from Costello *et al.* (1957). The histological procedures in the latter reference (page 225) were used with many variations. Flemming's seems to be a more satisfactory fixative, and an isopropyl dehydration and clearing can be substituted for the ethanol-amyl acetate procedure. The stain most frequently used was Ehrlich's hematoxylin and eosin. When it was desirable to see the earliest pigment granules, safranin in 50% ethanol was used to allow visualization of the nuclei and cell outline. Basophilia was judged by eye on

sections stained in pH 4 azure-B. Sections were cut at 6 μ, or thinner when better resolution was desired. Embryos to be examined whole were fixed in Stockard's (1915) fixative.

Since the pigment and propigment cells form a rather diffuse tissue, if they may be called a tissue at all, sectioned material is not ideal for observing them. Any plane of sectioning through the embryo would give good results for only a few of these flattened and irregularly shaped cells. A procedure was developed which allowed satisfactory observation of these cells in their entirety on the *Fundulus* yolk sac. The live embryo was laid on its side on a clean coverslip. The yolk sac was punctured on its top surface, and the yolk and fluid around the embryo drawn away with filter paper leaving the embryo flattened out on the coverslip. The coverslip with the embryo on top was set on an aluminum rod, the other end of

TABLE I

Zebra fish stage sequence at 28° C.

Stage No.	Age in hours	Distinguishing characteristics (pigment cell appearance)	Hisaoka and Battle stage
15	15	Blastopore closure	17
16	16+	Optic vesicles visible	17
17	18	Formation of first somites	17
18	21	Auditory placodes formed, ten somites	18
19	23	Cavity in central nervous system	18
20	27	Partial constriction of yolk mass (First pigment granules appear)	19
21	30	Motility of embryo (Visibly pigmented eye)	20
22	33	Beginning of heart beat (First body melanophores visible)	21
23	35	Circulation begins (Melanophores appear on yolk)	22
24	39	Circulation on yolk sac (Great increase in general pigment)	22
25	45	Prominent fin buds (Some expanded melanophores on body and yolk sac)	22

which was immersed in liquid nitrogen (dry ice-acetone mixture when liquid nitrogen was not available). The embryo was thus frozen almost instantaneously. Under a dissecting microscope the top layers of the frozen embryo were carefully shaved away with a sharp scalpel or razor blade. Ideally, a layer consisting of most of one side of the embryo but only a few cell layers thick could be left on the coverslip. This tissue was then instantaneously thawed and fixed by dropping the coverslip into acetic-alcohol (1:3). The fixative precipitates the protein so that the tissue adheres to the glass. It is subsequently treated as sectioned material.

Living embryos were observed and staged with a dissecting microscope having a highest magnification of 80×. A phase contrast microscope was used extensively. It was found that the Zeiss 40× oil phase objective made it possible to observe many intracellular details in the living fish embryos. A filar ocular micrometer was used to obtain measurements on fixed material.

At the time this study was begun, there was no set of normal developmental stages for the zebra fish and cunner. Oppenheimer's (1937) stages were used for *Fundulus*. Since there is no stage sequence available for the cunner, the observations presented here are based on the age of the embryos in hours at approximately 17° C. A modification of Oppenheimer's stages was developed for the zebra fish; however, they cover only the period extending from the closure of the blastopore to the complete differentiation of the earlier pigment cells, and are based as much as possible on the pigment cells themselves. Recently the development of the zebra fish has been described (Hisaoka and Battle, 1958), but the stages given are too coarse for the purposes of the present study. Table I presents the stages used herein and a comparison of them with those of Hisaoka and Battle.

Observations

The teleost neural crest

In the zebra fish, the melanoblasts begin to migrate before they become pigmented. The first melanin that can be seen is in the eye, and it is followed by a wave of melanogenesis progressing posteriorly. Since there are slight differences in the time of appearance of melanin and since the crest also shows some modification in the head region and in the tail bud, the sequence of events to be described will be concerned mainly with the trunk region.

At stage 15, the embryonic axis of the zebra fish is represented only by a swelling in the midline of the embryonic shield with some separation of the notochord (Fig. 1). As stage 16 arrives, there is a well developed neural keel in the brain region, while in the trunk the keel is represented by a ridge in the ectoderm above a well delimited notochord (Fig. 2). Figures 3 to 8 show the trunk region in stages 17 to 24. It will be seen that the neural primordium becomes progressively larger and more discrete. Initially the dorsal surface of the keel is broadly attached to the ectoderm (Figs. 3 and 4). This attachment narrows in stage 18 (Fig. 5), and the cells constituting it become loose in stage 18 to 19 (Fig. 6). It is these cells that apparently represent the neural crest of the zebra fish embryo. In Figure 7, early migrating cells can be seen leaving the crest, and the first pigment granules are forming in some of these cells. Figure 8 shows the paths of migration outlined by pigmented cells, most of which have now ceased migration.

The majority of the melanoblasts first migrate between the neural tube and the somites and then down onto the surface of the notochord. From there some of them follow the remnants of what has been referred to as the segmentation cavity, to the yolk sac. Most of the cells that have become heavily pigmented along this route cease their migration and are eventually associated with various internal organs. Another path of migration is between the ectoderm and the somites. The cells in this position are easily visible with the phase microscope (Figs. 9 and 10), and are the ones that form the larval pigment. The paths of migration are quite similar to those described for the higher vertebrates.

Cytologically these cells are not as desirable for the study of melanoblast differentiation as those of the *Fundulus* yolk sac which will be described in a later section; however, some of the same phenomena are observable. Prior to

Symbols Used in Figure Legends

B—Melanoblast
C—Notochord
E—Endothelial cell
N—Neural primordium
M—Melanophore
X—Neural crest

The scale marker is 10 μ long in all figures except Figures 17 and 23 where it is 100 μ.

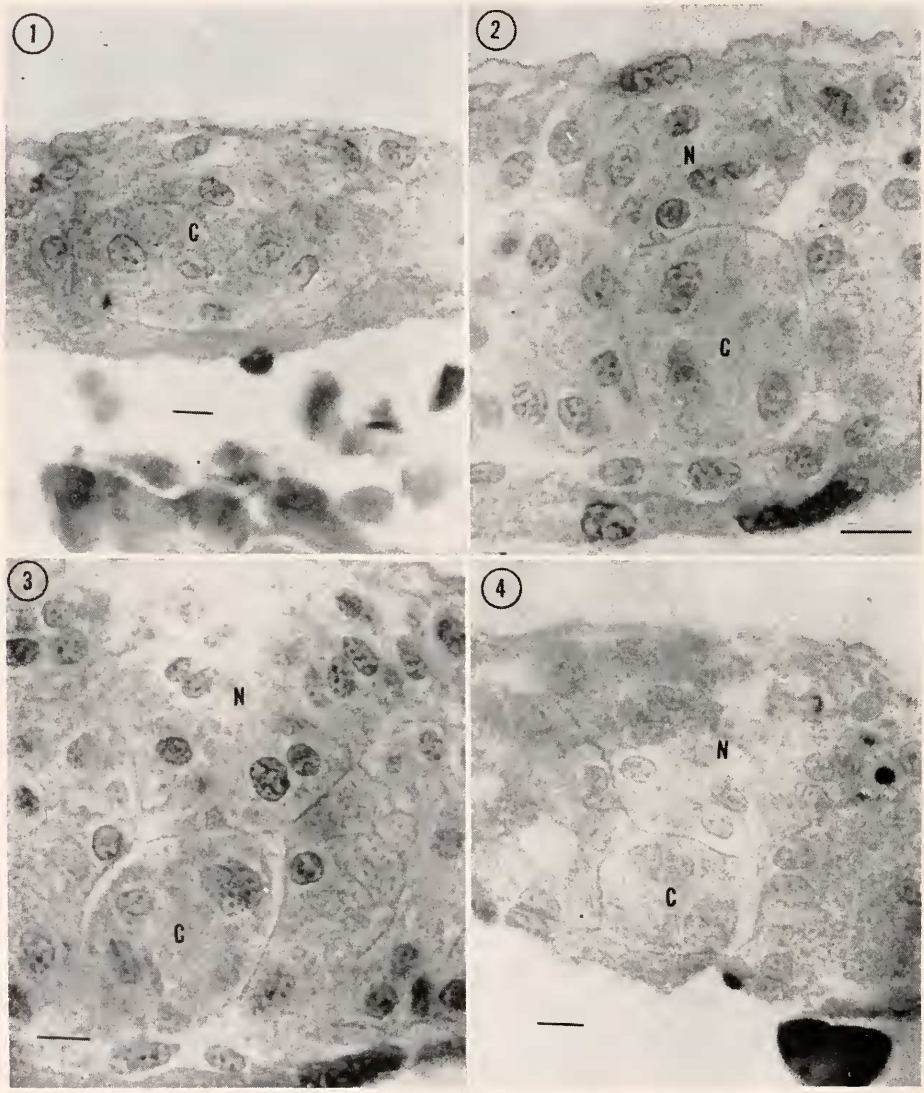

Figure 1. Zebra fish: cross-section at stage 15 showing the thickening in the midline of the embryo, which is the beginning of the neural primordium.

Figure 2. Zebra fish: cross-section at stage 16. The notochord is now distinct and the neural keel is beginning to form.

Figure 3. Zebra fish: cross-section at stage 17. The neural keel is enlarging.

Figure 4. Zebra fish cross-section at stage 17+. The keel is here quite large, but still broadly attached to the ectoderm.

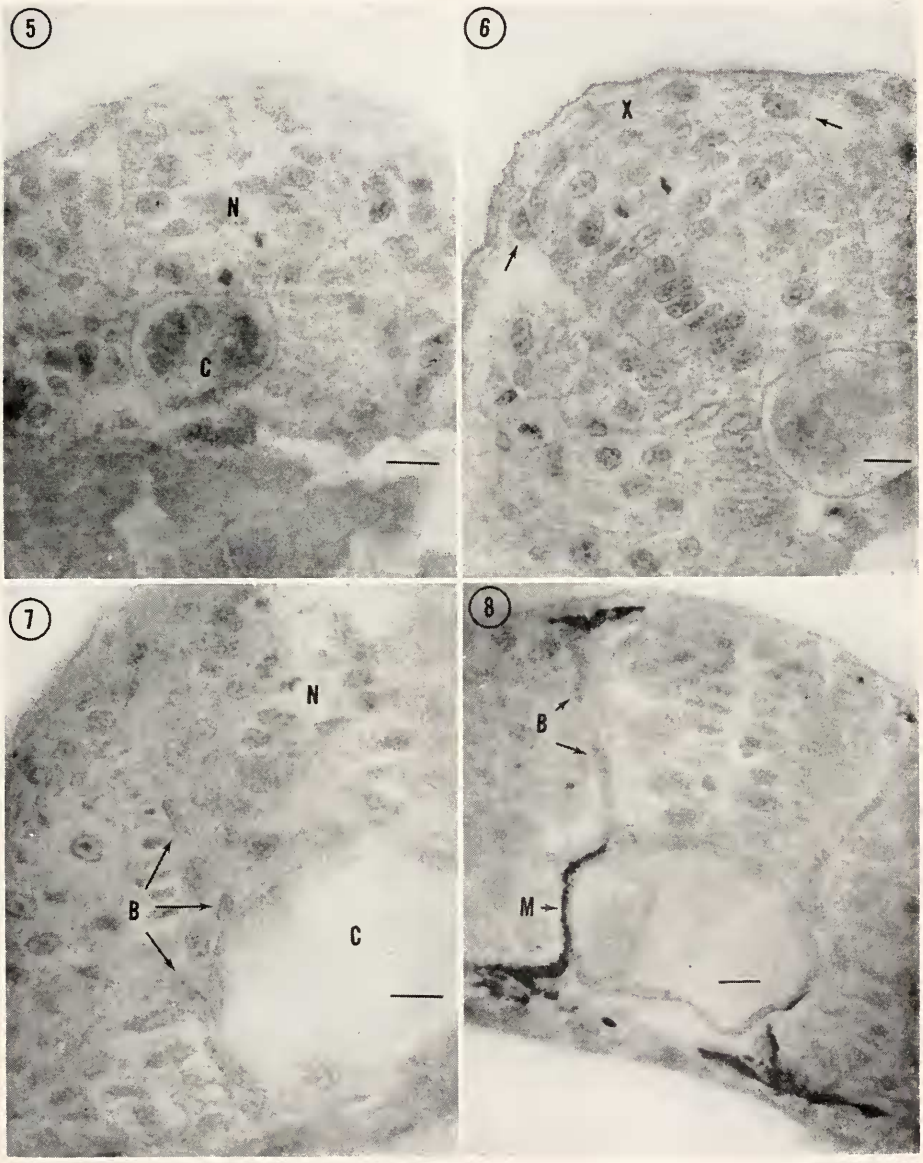

FIGURE 5. Zebra fish: cross-section at stage 18. The attachment of the keel to the ectoderm is becoming narrower.

FIGURE 6. Zebra fish: cross-section at stage 18+. This is the loose crest stage. The cells indicated (arrows) will migrate shortly.

FIGURE 7. Zebra fish: cross-section at stage 19. Some of the migrating cells (arrows) contain the first melanin granules.

FIGURE 8. Zebra fish: cross-section at stage 24. The main paths of pigment migration are outlined by melanophores.

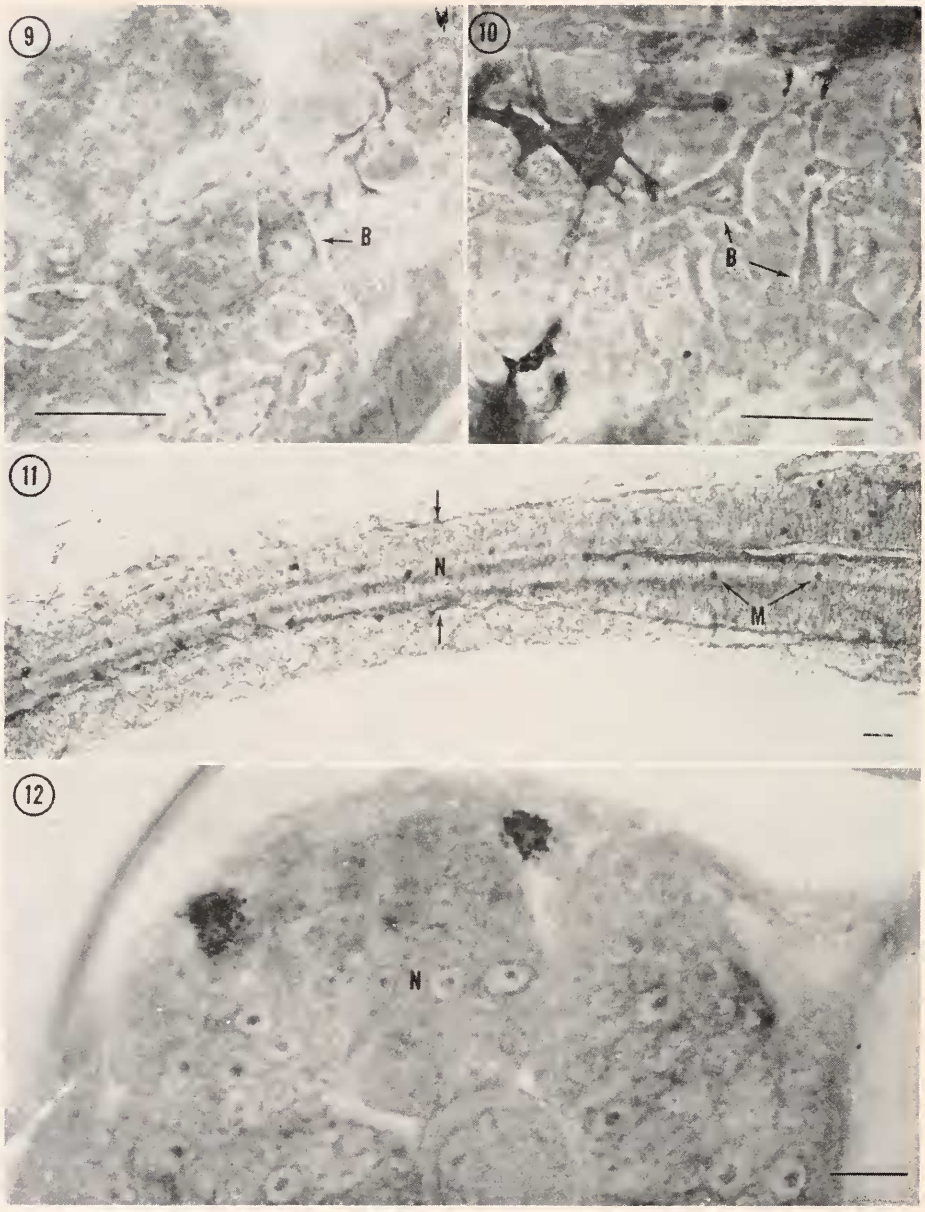

FIGURE 9. Zebra fish: phase photomicrograph at stage 24. Note the large nucleus and nucleolus of this melanoblast.

FIGURE 10. Zebra fish: phase photomicrograph at stage 24. This and the previous figure show cells in the intact, living embryo.

FIGURE 11. Dorsal aspect of a whole mount of a 30-hour cunner embryo. The width of the neural keel is indicated. The melanophores are confined to the area between the ectoderm and the keel.

FIGURE 12. Cunner: cross-section of a 20-hour embryo. Except for the presence of melanophores here, this closely resembles the loose crest stage of the zebra fish (Fig. 6).

melanogenesis there is a great increase in cytoplasmic basophilia. The nuclei triple or quadruple their volume during differentiation and contain large, densely basophilic nucleoli (Fig. 9). The cell size also increases enormously, although the very irregular shape (Fig. 10) makes calculations difficult. In general, the differentiation of the neural crest pigment cells in the zebra fish resembles that of the *Fundulus* yolk sac pigment which is not of neural crest derivation.

The pigment of the pelagic cunner egg is interesting because in similar embryos Orton (1953) and others have observed melanophores forming in the crest region well before any pigment cell migration. Figure 11 shows a 30-hour cunner embryo. The dorsal pigment has been visible for a number of hours, but the cells have not migrated. There is no pigment elsewhere in the embryo. The melanophores are still in close association with the neural tube which is beginning to form its cavity in the hind-brain region. A cross-section through an earlier cunner embryo (Fig. 12) shows the relationship of the melanophores to the neural keel. This stage resembles the loose crest stage of the zebra fish embryo.

The neural crest cells of *Fundulus* begin to migrate before melanogenesis occurs. Figures 13 to 16 show several stages in the development of the neural region of this form. By stage 14 the beginnings of the neural primordium are present. During stages 15 to 17 the keel develops rapidly (Figs. 13 and 14), and by late stage 16 or early 17 the crest becomes fairly distinct histologically. This stage may correspond with the loose crest stage of the zebra fish (Fig. 14). Between stages 17 or 18 and stage 21 crest migration begins in the trunk region (Fig. 15). Some crest migration may have started a little earlier in the head region, but it is difficult to distinguish head mesenchyme from crest cells. Sometimes at stage 18 early melanophores are visible in the head mesenchyme, possibly associated with the eye, but there are none in the trunk. Migration continues and melanization begins in the trunk during stages 19 and 20 (Fig. 16). In the discussion which follows, migrating cells are described on the yolk prior to stage 15. Some of these cells may be identified as melanoblasts at stage 16, and melanogenesis has begun in them by stage 18. Not until several hours later is the first melanin seen in the embryo proper. Thus, the yolk sac melanophores apparently are not derivatives of the neural crest.

The yolk sac melanophores

On the yolk sac of *Fundulus heteroclitus,* Stockard (1915) described wandering mesenchymal cells which eventually developed into four different cell types: black and brown pigment cells, red blood cells, and endothelial cells. These cells are readily observed with phase optics, and additional detail can be seen using the freezing-shaving technique described in this paper. The following description is based on these preparations.

The cells described by Stockard begin to migrate shortly before the closure of the blastopore (Oppenheimer stages 14 to 15) from the germ ring and possibly from the caudal mass and sides of the embryo. At these stages the cells are quite similar, and it is difficult to determine which types come from a given source. The migration continues until after the blastopore closes, at which time cells may be seen leaving the remnants of the germ ring (Fig. 17).

Stockard was not certain that his identification of these cells on the basis of

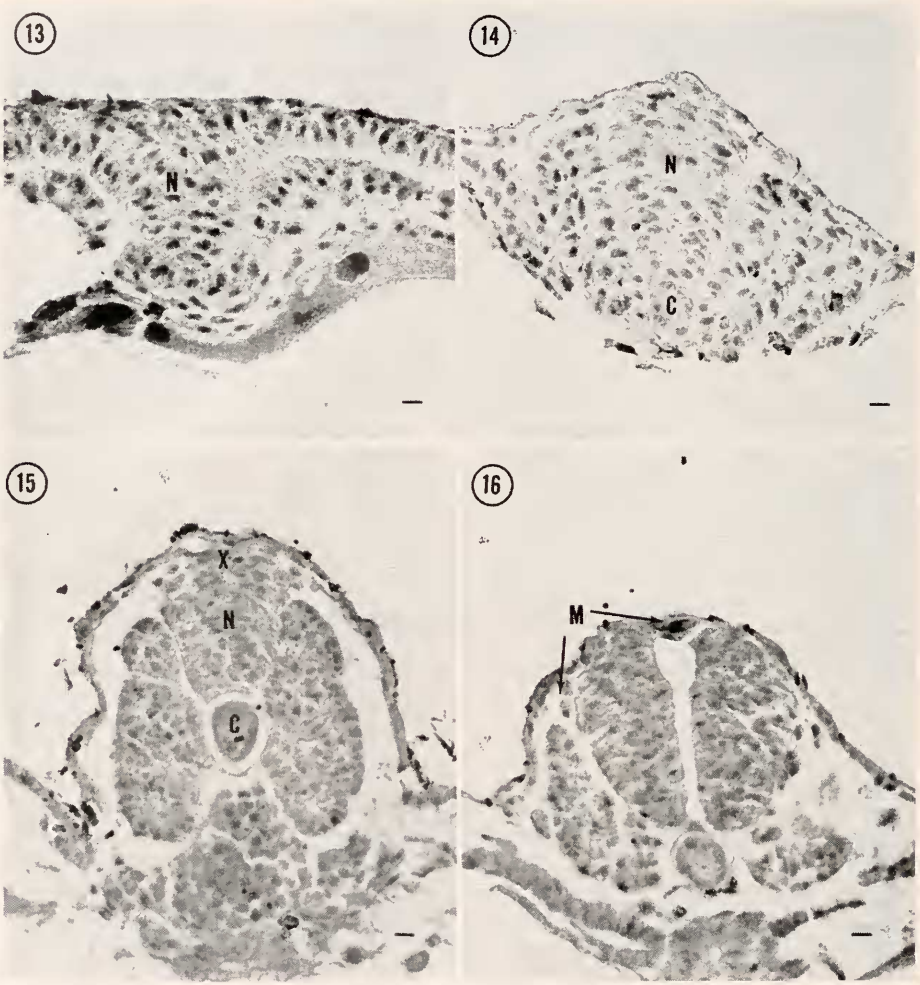

FIGURE 13. *Fundulus:* cross-section at stage 15. The neural keel is forming.

FIGURE 14. *Fundulus:* cross-section at stage 18. There is a well developed neural keel with loosening of the crest cells.

FIGURE 15. *Fundulus:* cross-section at stage 21. This is a late loose crest stage in the posterior trunk region.

FIGURE 16. *Fundulus:* cross-section at stage 21. The crest is migrating and melanophores are beginning to differentiate in this embryo. There are well developed yolk sac melanophores before this stage.

size and shape was fully warranted, and he suggested the possibility that some of the cells might be able to change from one type to another before their final differentiation. In the present study, however, it has been found that these wandering cells can be distinguished from each other on the basis of several cytological properties, and the changes in these characteristics were followed through the differentiation of the cells. (The brown pigment cells were not carefully

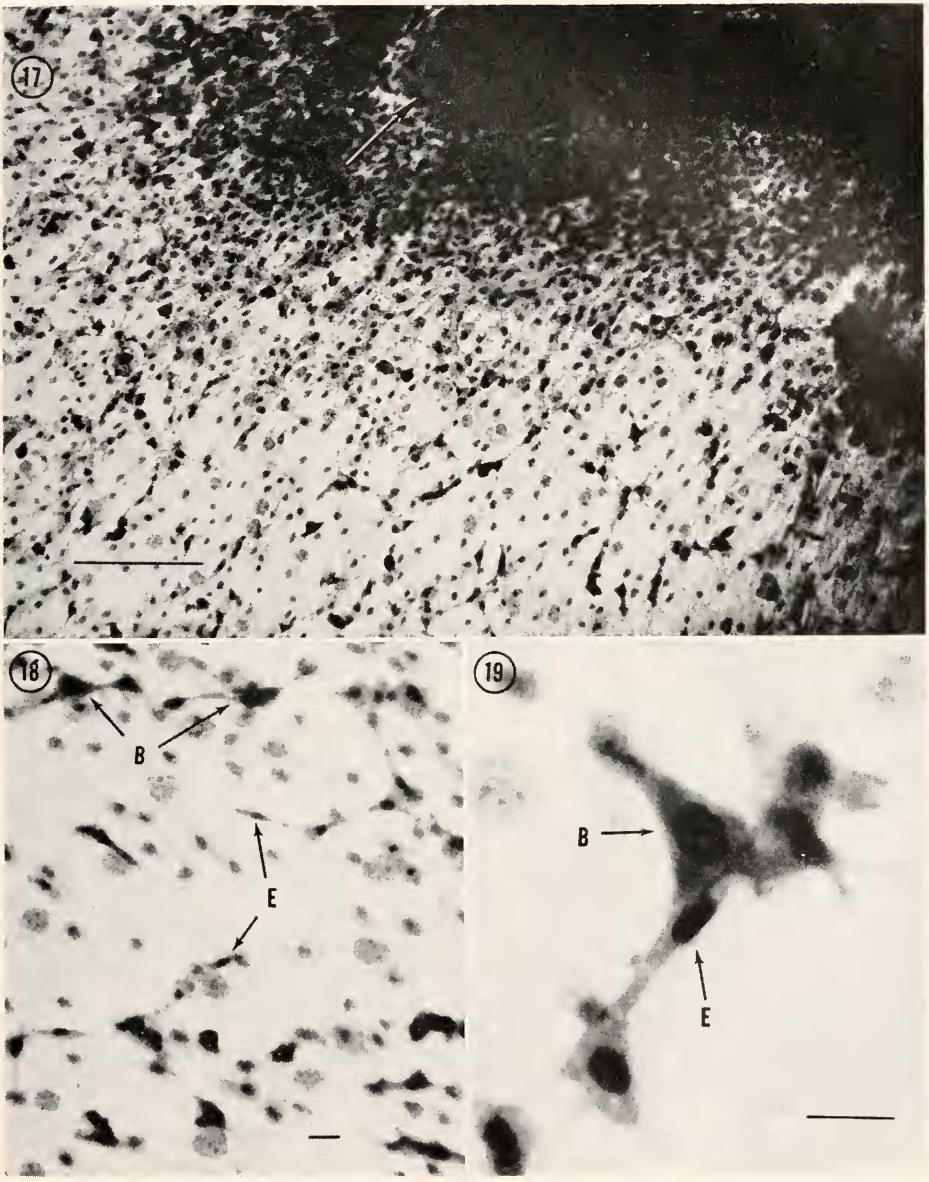

FIGURE 17. *Fundulus:* frozen-shaved preparation at stage 16. The caudal end of the embryo is at the arrow. The darkly stained cells are the wandering cells of Stockard while the lightly stained nuclei belong to the ectoderm. The scale line equals 100 μ.

FIGURE 18. *Fundulus:* frozen-shaved preparation at stage 16. The endothelial cells may be distinguished from the melanoblasts.

FIGURE 19. *Fundulus:* frozen-shaved preparation at stage 16. The large nucleus and nucleoli and the basophilic cytoplasm of the melanoblast are apparent here.

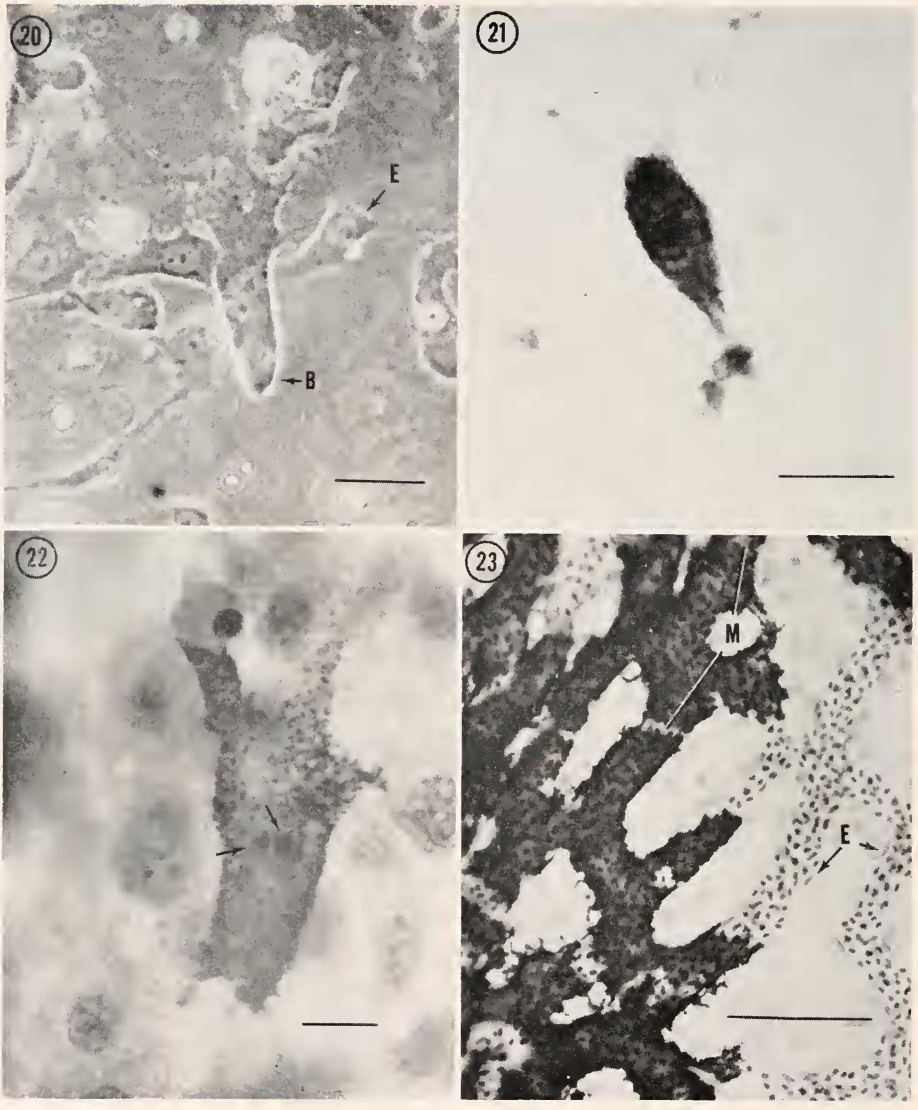

FIGURE 20. *Fundulus:* Phase photomicrograph of living stage 17 embryo. Note the cytoplasmic granularity of the melanoblast.

FIGURE 21. *Fundulus:* frozen-shaved preparation at stage 17+. Note the intense basophilia of this azure-B-stained melanoblast.

FIGURE 22. *Fundulus:* frozen-shaved preparation at stage 20. The nucleoli are still large in this early melanophore (arrows).

FIGURE 23. *Fundulus:* frozen-shaved preparation at stage 32. The extent of one melanophore is indicated. At stage 16 these cells were the same size as the blood and endothelial cells of the blood vessels which they now encompass. The scale mark is 100 μ.

followed. They seemed to arrive later and are possibly of neural crest origin.)

The migrating cells initially are spindle-shaped with lightly staining cytoplasm. Their nuclei are spherical, approximately 5 μ in diameter (70 μ^3 in volume), containing several small nucleoli. The endothelium and blood cells subsequently "lose" their nucleoli at stages 17 and 19, respectively. The blood cells become small and spherical with sharp, radiating pseudopods. They have a granular cytoplasm by stage 20, and there is little change in their nuclear volume. The endothelial cells retain their spindle shape, but stain less intensely than the original cells. Even after these cells expand to form the blood vessels of the yolk, their nuclei show little increase in volume.

The changes in the melanoblasts are quite striking, however. Figures 18 to 23 show stages in the differentiation of these cells. The most dramatic early changes are in the nuclei. The nucleoli increase in size and basophilia until the onset of melanogenesis (Figs. 19 to 22). At this time the nuclear volume is approximately 250 μ^3, and it increases further until in the fully differentiated melanophore it is almost 600 μ^3. This represents a nuclear volume increase of almost ten times over that of the initial cells. The cytoplasm of these cells becomes quite basophilic as compared with the other wandering cells (Fig. 21). A granularity also appears at stage 17 (Fig. 20), possibly representing propigment granules. The over-all cell size increases rapidly during differentiation until the final immense size of the expanded melanophore is reached (Fig. 23). These cells may thus be identified and followed with a fair degree of certainty from stage 16 through their final differentiation, and at stage 16 there is no sign of neural crest migration except rarely in the head region.

A brief search was made in the tropical fish stores and the streams of the Chicago area for other cyprinodont species which appeared to have the "non-neural crest" pigment. Simple observational criteria were used. (1) Are there numbers of relatively large wandering cells on the yolk sac before pigmentation begins? (2) Do melanophores appear evenly distributed over the yolk sac before the body pigment appears? (3) Do the yolk melanophores eventually wrap around the blood vessels of the yolk sac? The only species found which was affirmative for all three criteria was *Fundulus dispar*. *Epiplatys chaperi* was affirmative for the first two and was the only other species in which there appeared to be a secondary site of melanophore origin. Two species of *Amphyosemion* appeared to have neural crest pigment which migrated onto the yolk sac before melanogenesis began. Several other cyprinodonts had little or no yolk pigment, and no representative of the five other orders briefly studied satisfied any of the criteria. This sample, however, may be too small to make valid generalizations about the teleosts as a whole.

Discussion

In the higher classes of vertebrates where the neural tube is formed by neural folds, the neural crest might be viewed as the ectodermal tissue connecting the neural primordium with the general ectoderm. Although the neural keel of the teleosts is formed in a different manner, the crest occupies a position between it and the ectoderm. As the crest migrates, the dorsal border of the neural primordium becomes progressively more distinct until finally all attachment to the ectoderm is lost.

Since several investigators have demonstrated that the neural crest is the sole source of melanophores in most of the higher vertebrate classes (see DuShane, 1946), it is frequently stated that all vertebrate pigment cells have the same origin (see Rawles, 1948, p. 405). There is good evidence for the neural crest origin of pigment in the lampreys (Agnatha) (Newth, 1956) and fair evidence for the teleosts in general. As yet, however, there appears to be no evidence with regard to the Chondrichthyes or the Reptilia.

The relatively minor exception to the above generalization provided by *Fundulus* does, however, make a more thorough experimental study of the teleosts seem desirable. For lack of confirmation or completeness, none of the work cited constitutes adequate proof that the neural crest is the sole source of melanophores in any species of fish, while there appears to be good evidence in one species that some melanophores have a different origin.

The significance of the exception cannot be easily evaluated at present for several reasons. First, it is not known how frequently it occurs in other forms, although it does appear to be relatively rare. Secondly, the source of these exceptional melanophores has not been conclusively demonstrated, although any direct relationship with the neural crest is unlikely. Thirdly, the adaptive significance of the yolk sac pigment is unclear.

The first point above has been discussed elsewhere in this paper, and there is nothing more to add here. With regard to adaptive significance, if some fish species require either earlier pigment or pigment of different characteristics than that provided by the crest (see Rass, 1937), then the occurrence of such pigment may be of little general significance. If, instead, relatively undifferentiated tissues such as the germ ring have the potentiality of differentiating into melanophores (see Barth *et al.*, 1960), it might be found that many unrelated species have some non-neural crest pigment cells.

A certain amount of work has been done that bears on the second point above. Stockard states that most of the "wandering mesenchyme cells" come from the germ ring and its remnants after closure of the blastopore, and a smaller number come from the lateral sides of the embryo, especially at its caudal end. The differentiation of these cells does not take place until they are widely spread over the yolk sac, and it is difficult to say where they came from. Possibly they could be followed individually or traced by vital staining. Empirically, the germ ring seems the most likely source of the melanophores. It contributes most of the cells, and although there is disagreement in the literature concerning the fate of the germ ring (Lewis, 1912; Oppenheimer, 1938; Brummett, 1954), Oppenheimer's experiments demonstrate that it has broad potentialities for differentiation under experimental conditions. Perhaps it is simplest to consider the germ ring a totipotent remnant of the blastodisc which has felt comparatively little influence from the gastrulation of the embryo proper. That the embryo has little influence is borne out by the experiments of Trinkaus (1951) and by the fact that anomalous eggs may be found occasionally in which there are normal yolk sac membranes with melanophores, but no sign of embryonic structures.

It is interesting to note that the cytological aspects of differentiation are similar in both the neural crest and non-neural crest melanophores, and that in both the signs of greatest cellular activity precede the onset of melanogenesis. Quantitative

studies of cytoplasmic basophilia or nucleolar basophilia and size would be expected to follow much the same curve as the one for oxygen consumption given by Flickinger (1949)—reaching a peak at the onset of melanogenesis and falling off thereafter. Such data tend to indicate that in the melanophore, at least, the processes of differentiation may take more cellular "effort" than the elaboration of the materials characteristic of the differentiated cell.

Much of the material in this paper was submitted as a dissertation for the degree of Master of Science at the University of Chicago. The author wishes to express his thanks to his sponsor, Dr. Hewson Swift, for help on many aspects of this study.

Summary

In this study, three species of fish which differ in the developmental histories of their pigment cells have been used—the zebra fish (*Brachydanio rerio*), the cunner (*Tautogolabrus adspersus*), and *Fundulus heteroclitus*. Their melanophores have been studied with respect to their origins and subsequent histories, including some of the cytological events occurring during differentiation.

1. In the teleosts, the neural crest has been identified as the tissue connecting the neural keel with the overlying ectoderm. During development, this tissue loosens, and the cells migrate from this region. In some of these cells melanogenesis has already begun.

2. Differentiated melanophores are demonstrated in the neural crest of the cunner embryo well before migration occurs.

3. It is concluded that the normal embryonic pigment of the teleosts has its origin in the neural crest, as in other vertebrates.

4. In some fish, notably in *Fundulus*, the pigment cells of the yolk sac are not of neural crest origin. Instead they appear to arise from the extra-embryonic germ ring. These cells may be identified on the yolk sac several hours before there is histological evidence of crest migration.

5. Many of the cytological aspects of differentiation are similar in the melanophores, regardless of their site of origin.

LITERATURE CITED

Axelrod, H. R., and L. P. Schultz, 1955. Handbook of Tropical Aquarium Fishes. McGraw-Hill Co., New York.

Bancroft, F. W., 1912. Heredity of pigmentation in *Fundulus* hybrids. *J. Exp. Zool.*, **12**: 153–178.

Barth, L. J., L. G. Barth and I. Nelson, 1960. Induction of pigment cells from the presumptive epidermis of *Rana pipiens* gastrula. *Anat. Rec.*, **137**: 337–338.

Borcéa, M. I., 1909. Sur l'origine du coeur, des cellules vasculaires migratrices et des cellules pigmentaires chez les téléostans. *C. R. Acad. Sci., Paris*, **149**: 688–689.

Brummett, A. R., 1954. The relationship of the germ ring to the formation of the tail bud in *Fundulus*. *J. Exp. Zool.*, **125**: 447–481.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox and C. Henley, 1957. Methods for Obtaining and Handling Marine Eggs and Embryos. Marine Biological Laboratory, Woods Hole, Mass.

DuShane, G. P., 1935. An experimental study of the origin of pigment cells in Amphibia. *J. Exp. Zool.*, **72**: 1–31.

DuShane, G. P., 1946. The development of pigment cells in vertebrates. *In:* Biology of the Melanomas. *Spec. Publ. N. Y. Acad. Sci.*, **4**: 1–15.

Flickinger, R. E., 1949. A study of the metabolism of the amphibian neural crest cells during their migration and pigmentation *in vitro*. *J. Exp. Zool.*, **112**: 465–484.

Gilson, A. S., 1926. Melanophores in developing and adult *Fundulus*. *J. Exp. Zool.*, **45**: 415–457.

Goodrich, H. B., 1950. Problems of origin and migration of pigment cells in fish. *Zoologica*, **35**: 17–19.

Hisaoka, H. K., and H. I. Battle, 1958. The normal stages of the zebra fish. *J. Morphol.*, **102**: 311–321.

Humm, D. G., and R. S. Young, 1956. The embryological origin of pigment cells in platyfish-swordtail hybrids. *Zoologica*, **41**: 1–10.

Kajishima, T., 1958. Regional differences in pigment cell formation of the embryonic shield of the goldfish. *Embryologia*, **4**: 133–147.

Lewis, W. H., 1912. Experiments on localization and regeneration in the embryonic shield and germ ring of a teleost fish, *Fundulus*. *Anat. Rec.*, **6**: 325–334.

Lopashov, G. V., 1944. Origin of pigment cells and visceral cartilages in teleosts. *C. R. (Doklady) Acad. Sci. U. R. S. S.*, **44**: 169–172.

Newth, D. R., 1951. Experiments on the neural crest of the lamprey embryo. *J. Exp. Biol.*, **28**: 247–260.

Newth, D. R., 1956. On the neural crest of the lamprey embryo. *J. Embryol. Exp. Morphol.*, **4**: 358–375.

Oppenheimer, J. M., 1937. The normal stages of *Fundulus heteroclitus*. *Anat. Rec.*, **68**: 1–15.

Oppenheimer, J. M., 1938. Potencies for differentiation in the teleostean germ ring. *J. Exp. Zool.*, **79**: 185–212.

Oppenheimer, J. M., 1949. Atypical pigment cell differentiation in embryonic teleostean grafts and isolates. *Proc. Nat. Acad. Sci.*, **35**: 709–712.

Orton, G. L., 1953. Development and migration of pigment cells in some teleost fishes. *J. Morphol.*, **93**: 69–89.

Rass, T. S., 1937. Pigmentation of embryos and larvae in the herrings as an adaptation to a pelagic mode of life. *Bull. Soc. Nat. Moscow, N. S.*, **46**: 163–164.

Rawles, M. E., 1948. Origin of melanophores and their role in the development of color patterns in vertebrates. *Physiol. Rev.*, **28**: 383–408.

Russel, A., 1939. Pigment inheritance in *Fundulus-Scomber* hybrids. *Biol. Bull.*, **77**: 423–431.

Stockard, C. R., 1915. A study of wandering mesenchymal cells on the living yolk sac and their developmental products: chromatophores, vascular endothelium, and blood cells. *Amer. J. Anat.*, **18**: 525–594.

Trinkaus, J. P., 1951. Analysis of blastoderm expansion of the egg of *Fundulus heteroclitus*. *Anat. Rec.*, **111**: 550–551.